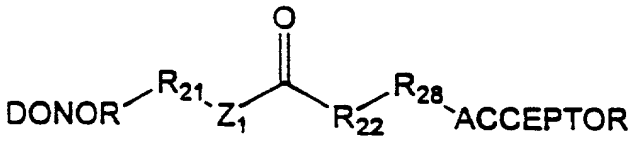
CLAIMS

WHAT IS CLAIMED IS:

1. An energy transfer dye having the structure



where

DONOR is a dye capable of absorbing light at a first wavelength and emitting excitation energy in response;

ACCEPTOR is dye which is capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response;

C(O) is a carbonyl group;

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

R_{21} is a C_{1-5} alkyl attached to the donor dye;

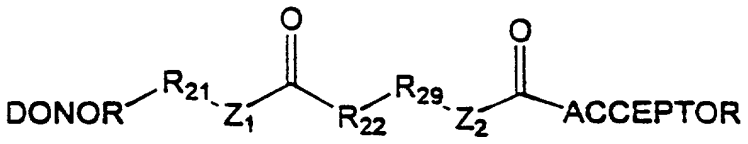
R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond or a fused ring structure which is attached to the carbonyl carbon; and

R_{28} includes a functional group which attaches the linker to the acceptor dye.

2. The energy transfer dye according to claim 1 wherein R_{22} is a five or six membered ring selected from the group consisting of cyclopentene, cyclohexene, cyclopentadiene, cyclohexadiene, furan, thiofuran, pyrrole, isopyrole, isoazole, pyrazole, isoimidazole, pyran,

pyrone, benzene, pyridine, pyridazine, pyrimidine, pyrazine oxazine, indene, benzofuran, thionaphthene, indole and naphthalene.

3. The energy transfer dye according to claim 1 wherein the linker has the structure

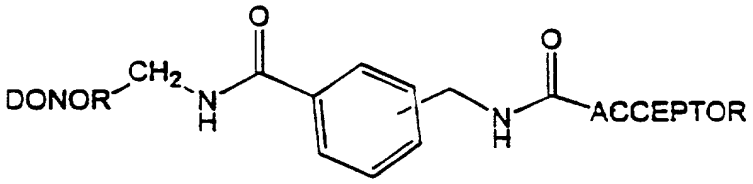


wherein

Z_2 is selected from the group consisting of NH, sulfur and oxygen; and

R_{29} is a C_{1-5} alkyl.

4. The energy transfer dye according to claim 1 wherein the linker has the structure



5. The energy transfer dye according to claim 1 wherein the donor dye is a member of the xanthene class of dyes.

6. The energy transfer dye according to claim 5 wherein the acceptor dye is a member of a class of dyes selected from the group consisting of xanthene, cyanine, phthalocyanine and squaraine dyes.

7. The energy transfer dye according to claim 1 wherein the donor dye is a member of a class of dyes selected from the group consisting of fluorescein, rhodamine and asymmetric benzoxanthene dyes.

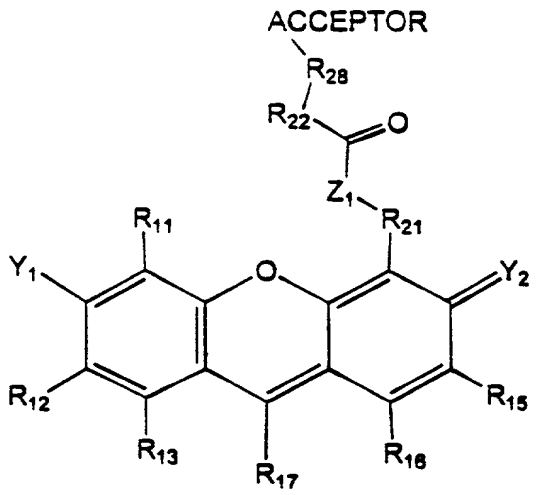
8. The energy transfer dye according to claim 7 wherein the acceptor dye is a member of a class of dyes selected from the group consisting of xanthene, cyanine, phthalocyanine and squaraine dyes.

9. The energy transfer dye according to claim 1 wherein the donor dye is selected from the group consisting of carboxyfluoresceins, 4,7-dichlorofluorescein dyes, asymmetric benzoxanthene dyes, rhodamine, 4,7-dichlororhodamine dyes, carboxyrhodamines, N,N,N',N'-tetramethyl carboxyrhodamines, carboxy R110, and carboxy R6G.

10. The energy transfer dye according to claim 1 wherein the acceptor dye is selected from the group consisting of 4,7-dichlorofluorescein dyes, asymmetric benzoxanthene dyes, rhodamine, 4,7-dichlororhodamine dyes, carboxyrhodamines, N,N,N',N'-tetramethyl carboxyrhodamines, carboxy R110, carboxy R6G, carboxy-X-rhodamines and Cy5.

11. The energy transfer dye according to claim 1 wherein the acceptor dye is selected from the group consisting DR110-2, DR6G-2, DTMR and DROX.

12. An energy transfer dye having the structure



where

C(O) is a carbonyl group;

Y_1 and Y_2 are each independently selected from the group consisting of hydroxyl, oxygen, iminium and amine;

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

R_{11}-R_{17} are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, phenyl, substituted phenyl, where adjacent substituents are taken together to form a ring, and combinations thereof;

R_{21} is a C_{1-5} alkyl;

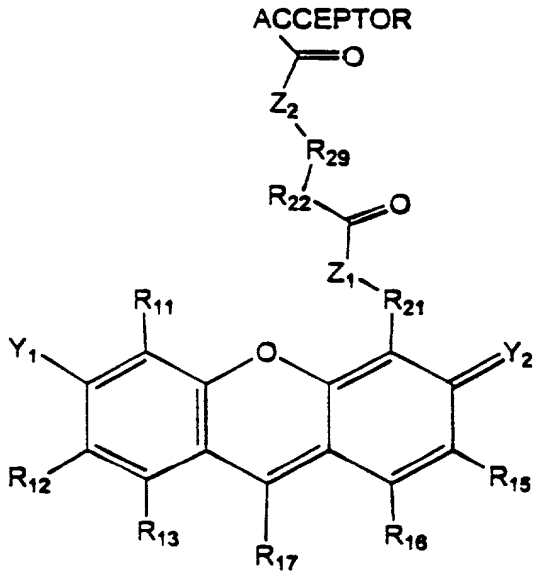
R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond or a fused ring structure which is attached to the carbonyl carbon;

R_{28} includes a functional group which attaches the linker to the acceptor dye; and

ACCEPTOR is dye which is capable of absorbing excitation energy emitted by a member of the xanthene class of dyes.

13. The energy transfer dye according to claim 12 wherein R_{22} is a five or six membered ring selected from the group consisting of cyclopentene, cyclohexene, cyclopentadiene, cyclohexadiene, furan, thiofuran, pyrrole, isopyrole, isoazole, pyrazole, isoimidazole, pyran, pyrone, benzene, pyridine, pyridazine, pyrimidine, pyrazine oxazine, indene, benzofuran, thionaphthene, indole and naphthalene.

14. The energy transfer dye according to claim 12 wherein the dye has the structure

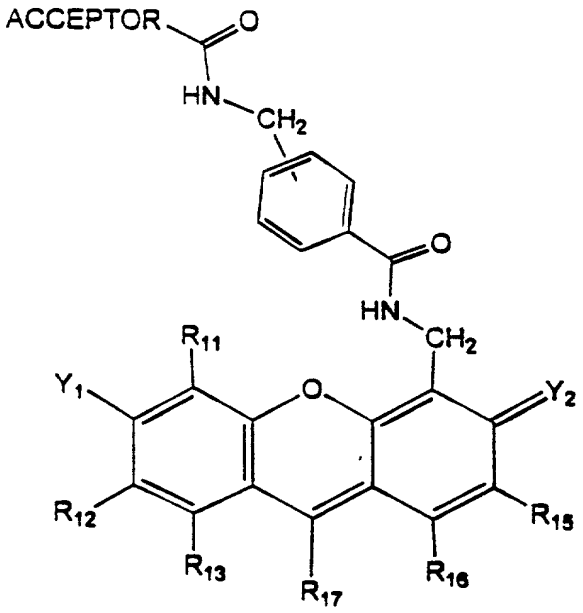


wherein

Z_2 is selected from the group consisting of NH, sulfur and oxygen; and

R_{29} is a C_{1-5} alkyl.

15. The energy transfer dye according to claim 12 wherein the linker has the structure



16. The energy transfer dye according to claim 12 wherein the acceptor dye is a member of a class of dyes selected from the group consisting of xanthene, cyanine, phthalocyanine and squaraine dyes.

17. The energy transfer dye according to claim 12 wherein the donor dye is a member of a class of dyes selected from the group consisting of fluorescein, rhodamine and asymmetric benzoxanthene dyes.

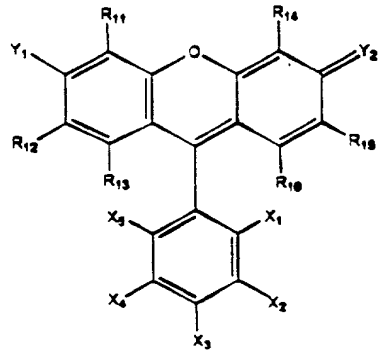
18. The energy transfer dye according to claim 17 wherein the acceptor dye is a member of a class of dyes selected from the group consisting of xanthene, cyanine, phthalocyanine and squaraine dyes.

19. The energy transfer dye according to claim 12 wherein the donor dye is selected from the group consisting of carboxyfluoresceins, 4,7-dichlorofluorescein dyes, asymmetric benzoxanthene dyes, rhodamine, carboxyrhodamines, N,N,N',N'-tetramethyl carboxyrhodamines, carboxy R110, and carboxy R6G.

20. The energy transfer dye according to claim 19 wherein the acceptor dye is a member of a class of dyes selected from the group consisting of xanthene, cyanine, phthalocyanine and squaraine dyes.

21. The energy transfer dye according to claim 12 wherein the acceptor dye is selected from the group consisting of 4,7-dichlorofluorescein dyes, asymmetric benzoxanthene dyes, rhodamine, 4,7-dichlororhodamine dyes, carboxyrhodamines, N,N,N',N'-tetramethyl carboxyrhodamines, carboxy R110, carboxy R6G, carboxy-X-rhodamines and Cy5.

22. The energy transfer dye according to claim 12 wherein the acceptor has the general structure



wherein:

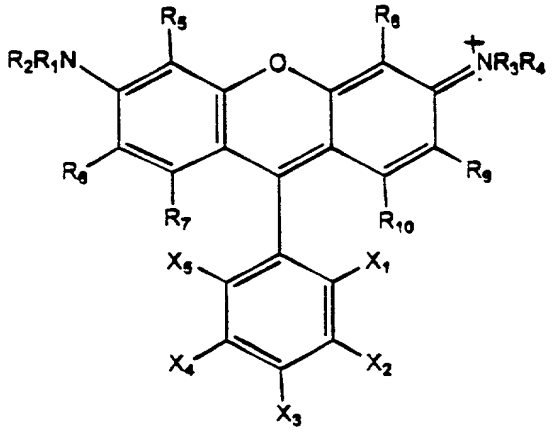
Y_1 and Y_2 are each independently selected from the group consisting of hydroxyl, oxygen, iminium and amine;

R_{11}-R_{18} are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, phenyl, substituted phenyl, where adjacent substituents are taken together to form a ring, and combinations thereof;

X_1 - X_5 are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, where adjacent substituents are taken together to form a ring, and combinations thereof; and

one of X_3 and X_4 is attached to the R_{28} group.

23. The energy transfer dye according to claim 12 wherein the acceptor dye has the general structure



wherein:

R_1-R_4 are each independently selected from the group consisting of hydrogen, and alkyl or where one or more of the groups of R_1 and R_5, R_2 and R_6, R_3 and R_8, R_4 and R_9 are taken together to form a ring;

R_5-R_{10} are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile, alkoxy, phenyl, and substituted phenyl, or where two or more of R_5-R_{10} are taken together to form one or more rings;

X_1, X_3 and X_4 are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile, or alkoxy;

X_2 and X_5 are chlorine; and

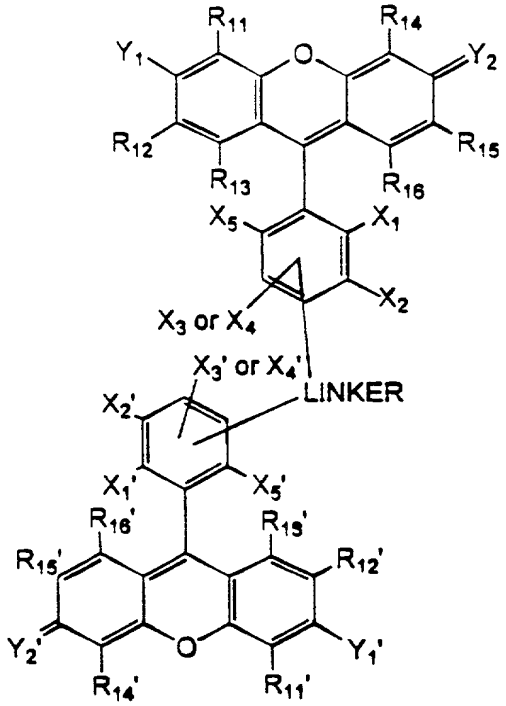
one of X_3 and X_4 are attached to R_{28}.

24. The energy transfer dye according to claim 23 wherein the rings formed by substituents R_5-R_{10} are 5, 6 or 7 membered rings.

25. The energy transfer dye according to claim 23 wherein one or more of the groups of R_1 and R_5, R_2 and R_6, R_3 and R_8, R_4 and R_9 are taken together to form a 5, 6 or 7 membered ring.

26. The energy transfer dye according to claim 23 wherein R_1 - R_{10}, X_1, X_3 and X_4 are selected to correspond to a dye selected from the group consisting of DR110-2, DR6G-2, DTMR-2, and DROX-2.

27. An energy transfer fluorescent dye having the general structure



wherein:

Y_1, Y_1', Y_2 and Y_2' are each independently selected from the group consisting of hydroxyl, oxygen, iminium and amine,

R_{11} - R_{16} and R_{11}' - R_{16}' are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, phenyl, substituted phenyl, where adjacent substituents are taken together to form a ring, and combinations thereof, and

X_1 - X_5 and X_1 ' - X_5' are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido,

nitrile, alkoxy, where adjacent substituents are taken together to form a ring, and combinations thereof;

Y_1, Y_2, R_{11} - R_{16}, and X_1 - X_5 are selected to correspond to a donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response;

Y_1', Y_2', R_{11}' - R_{16}', and X_1' - X_5' are selected to correspond to an acceptor dye which is capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response; and

one of X_3 and X_4 and one of X_3' and X_4' are taken together to form a linker linking the donor to the acceptor dye such that energy is transferred from the donor to the acceptor dye.

28. The energy transfer dye according to claim 27 wherein the linker has a backbone attaching the donor to the acceptor which is less than 9 atoms in length.

29. The energy transfer dye according to claim 27 wherein the linker has the general formula $R_{25}Z_3C(O)$ where R_{25} is a C_{1-4} alkyl attached to the donor dye at the X_3 or X_4 substituent, Z_3 is either NH, O or S, C(O) is a carbonyl group and the terminal carbonyl group is attached to the acceptor dye at the X_3' or X_4' substituent.

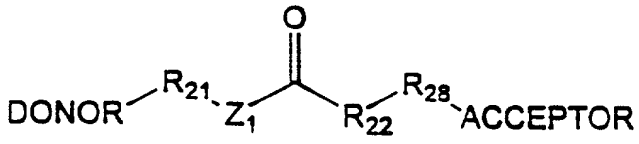
30. The energy transfer dye according to claim 27 wherein the linker has the general formula $R_{25}Z_3C(O)R_{26}Z_4C(O)$ where R_{25} is a C_{1-4} alkyl attached to the donor dye at the X_3 or X_4 substituent, R_{26} is a C_{1-4} alkyl, Z_3 and Z_4 are each independently either NH, O or S, C(O) is a carbonyl group and the terminal carbonyl group is attached to the acceptor dye at the X_3' or X_4' substituent.

31. An energy transfer fluorescent dye selected from the group consisting of: 5 or 6 carboxy TMR-B-CF, 5 or 6 carboxy TMR-F-CF, 5 or 6 carboxy TMR-P-CF, 5 or 6 carboxy TMR-P-CF, 5 or 6 carboxy TMR-A-CF, 5 or 6 carboxy TMR-D-CF, 5 or 6 carboxy TMR-N-CF, 5 or 6 carboxy ROX-CF, CY5-CF, 5 or 6 carboxy TMR-gly-5AMF and 5 or 6 carboxy TMR-5AMF, 5 or 6 carboxy CF-B-TMR-2, 5 or 6 carboxy CFB-DR110-2, 5 or 6 carboxy CFB-DR6g-2, and 5 or 6 carboxy CFB-DROX-2.

32. A fluorescently labeled reagent comprising:

a reagent selected from the group consisting of a nucleoside, nucleoside monophosphate, nucleoside diphosphate, nucleoside triphosphate, oligonucleotide and oligonucleotide analog, modified to be linked to an energy transfer fluorescent dye; and

an energy transfer fluorescent dye attached to the reagent, the energy transfer fluorescent dye including a dye having the structure



where

DONOR is a dye capable of absorbing light at a first wavelength and emitting excitation energy in response;

ACCEPTOR is dye which is capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response;

C(O) is a carbonyl group;

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

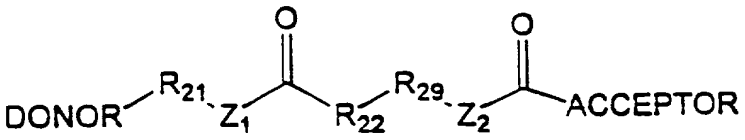
R_{21} is a C_{1-5} alkyl attached to the donor dye;

R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond or a fused ring structure which is attached to the carbonyl carbon; and

R_{28} includes a functional group which attaches the linker to the acceptor dye.

33. The fluorescently labeled reagent according to claim 32 wherein R_{22} is a five or six membered ring selected from the group consisting of cyclopentene, cyclohexene, cyclopentadiene, cyclohexadiene, furan, thiofuran, pyrrole, isopyrole, isoazole, pyrazole, isoimidazole, pyran, pyrone, benzene, pyridine, pyridazine, pyrimidine, pyrazine oxazine, indene, benzofuran, thionaphthene, indole and naphthalene.

34. The fluorescently labeled reagent according to claim 32 wherein the linker has the structure

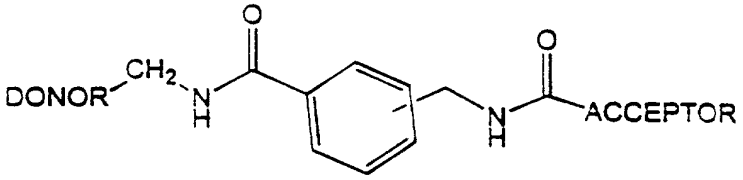


wherein

Z_2 is selected from the group consisting of NH, sulfur and oxygen; and

R_{29} is a C_{1-5} alkyl.

35. The fluorescently labeled reagent according to claim 32 wherein the linker has the structure



36. The fluorescently labeled reagent according to claim 32 wherein the donor dye is a member of the xanthene class of dyes.

37. The fluorescently labeled reagent according to claim 36 wherein the acceptor dye is a member of a class of dyes selected from the group consisting of xanthene, cyanine, phthalocyanine and squaraine dyes.

38. The fluorescently labeled reagent according to claim 32 wherein the donor dye is a member of a class of dyes selected from the group consisting of fluorescein, rhodamine and asymmetric benzoxanthene dyes.

39. The fluorescently labeled reagent according to claim 32 wherein the donor dye is selected from the group consisting of carboxyfluoresceins, 4,7-dichlorofluorescein dyes, asymmetric benzoxanthene dyes, rhodamine, 4,7-dichlororhodamine dyes, carboxyrhodamines, N,N,N',N'-tetramethyl carboxyrhodamines, carboxy R110, and carboxy R6G.

40. The fluorescently labeled reagent according to claim 32 wherein the acceptor dye is selected from the group consisting of 4,7-

dichlorofluorescein dyes, asymmetric benzoxanthene dyes, rhodamine, 4,7-dichlororhodamine dyes, carboxyrhodamines, N,N,N',N'-tetramethyl carboxyrhodamines, carboxy R110, carboxy R6G, carboxy-X-rhodamines and Cy5.

41. The fluorescently labeled reagent according to claim 32 wherein the acceptor dye is selected from the group consisting DR110-2, DR6G-2, DTMR and DROX.

42. The fluorescently labeled reagent according to claim 32 wherein the reagent is selected from the group consisting of deoxynucleoside, deoxynucleoside monophosphate, deoxynucleoside diphosphate and deoxynucleoside triphosphate.

43. The fluorescently labeled reagent according to claim 42 wherein the deoxynucleotides are selected from the group consisting of deoxycytosine, deoxyadenosine, deoxyguanosine, and deoxythymidine.

44. The fluorescently labeled reagent according to claim 32 wherein the reagent is selected from the group consisting of dideoxynucleoside, dideoxynucleoside monophosphate, dideoxynucleoside diphosphate and dideoxynucleoside triphosphate.

45. The fluorescently labeled reagent according to claim 32 wherein the dideoxynucleotides are selected from the group consisting of deoxycytosine, deoxyadenosine, deoxyguanosine, and deoxythymidine.

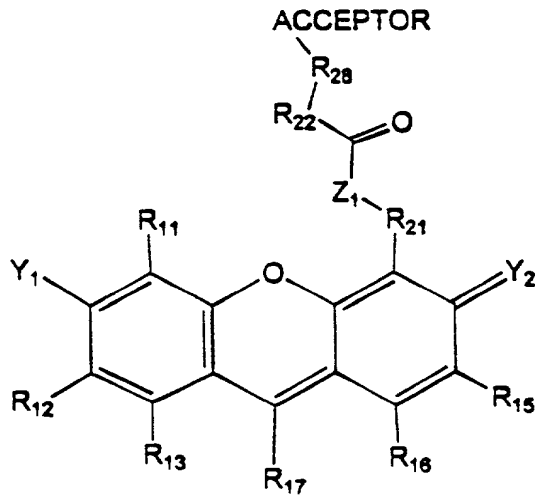
46. The fluorescently labeled reagent according to claim 42 wherein the reagent is an oligonucleotide.

47. The fluorescently labeled reagent according to claim 46 wherein the oligonucleotide has a 3' end which is extendable by using a polymerase.

48. A fluorescently labeled reagent comprising:

a reagent selected from the group consisting of a nucleoside, nucleoside monophosphate, nucleoside diphosphate, nucleoside triphosphate, oligonucleotide and oligonucleotide analog, modified to be linked to an energy transfer fluorescent dye; and

an energy transfer fluorescent dye attached to the reagent, the energy transfer fluorescent dye including a dye having the structure



where

C(O) is a carbonyl group;

Y_1 and Y_2 are each independently selected from the group consisting of hydroxyl, oxygen, iminium and amine;

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

R_{11}-R_{17} are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, phenyl, substituted phenyl, where adjacent substituents are taken together to form a ring, and combinations thereof;

R_{21} is a C_{1-5} alkyl;

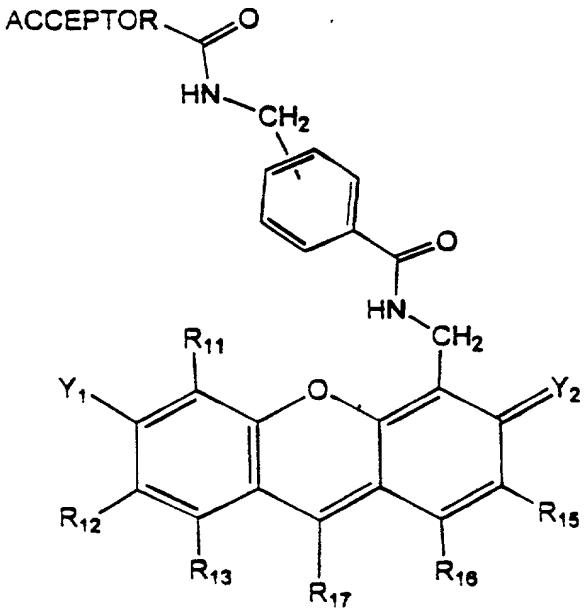
R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond or a fused ring structure which is attached to the carbonyl carbon;

R_{28} includes a functional group which attaches the linker to the acceptor dye; and

ACCEPTOR is dye which is capable of absorbing excitation energy emitted by a member of the xanthene class of dyes.

49. The fluorescently labeled reagent according to claim 48 wherein R_{22} is a five or six membered ring selected from the group consisting of cyclopentene, cyclohexene, cyclopentadiene, cyclohexadiene, furan, thiofuran, pyrrole, isopyrole, isoazole, pyrazole, isoimidazole, pyran, pyrone, benzene, pyridine, pyridazine, pyrimidine, pyrazine oxazine, indene, benzofuran, thionaphthene, indole and naphthalene.

50. The fluorescently labeled reagent according to claim 48 wherein the dye has the structure

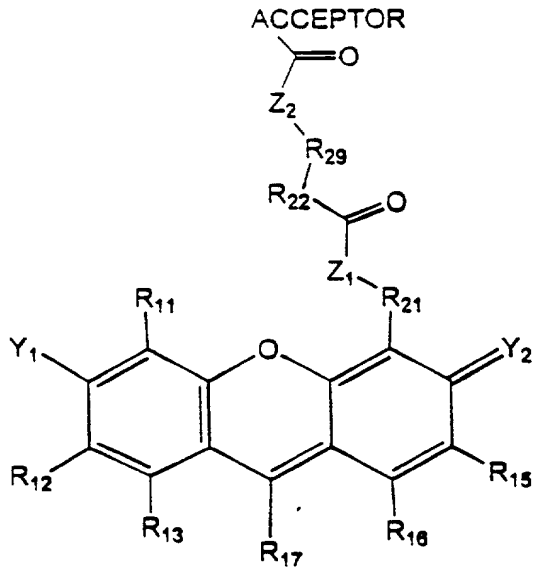


wherein

Z_2 is selected from the group consisting of NH, sulfur and oxygen; and

R_{29} is a C_{1-5} alkyl.

51. The fluorescently labeled reagent according to claim 48 wherein the linker has the structure



52. The fluorescently labeled reagent according to claim 48 wherein the acceptor dye is a member of a class of dyes selected from the group consisting of xanthene, cyanine, phthalocyanine and squaraine dyes.

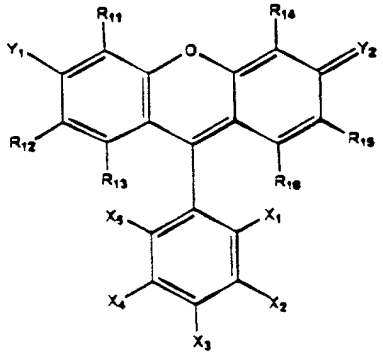
53. The fluorescently labeled reagent according to claim 48 wherein the donor dye is a member of a class of dyes selected from the group consisting of fluorescein, rhodamine and asymmetric benzoxanthene dyes.

54. The fluorescently labeled reagent according to claim 53 wherein the acceptor dye is a member of a class of dyes selected from the group consisting of xanthene, cyanine, phthalocyanine and squaraine dyes.

55. The fluorescently labeled reagent according to claim 48 wherein the donor dye is selected from the group consisting of carboxyfluoresceins, 4,7-dichlorofluorescein dyes, asymmetric benzoxanthene dyes, rhodamine, carboxyrhodamines, N,N,N',N'-tetramethyl carboxyrhodamines, carboxy R110, and carboxy R6G.

56. The fluorescently labeled reagent according to claim 48 wherein the acceptor dye is selected from the group consisting of 4,7-dichlorofluorescein dyes, asymmetric benzoxanthene dyes, rhodamine, 4,7-dichlororhodamine dyes, carboxyrhodamines, N,N,N',N'-tetramethyl carboxyrhodamines, carboxy R110, carboxy R6G, carboxy-X-rhodamines and Cy5.

57. The fluorescently labeled reagent according to claim 48 wherein the acceptor has the general structure



wherein:

Y_1 and Y_2 are each independently selected from the group consisting of hydroxyl, oxygen, iminium and amine;

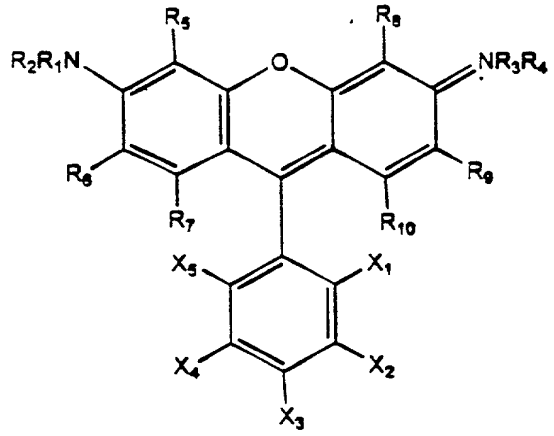
R_{11}-R_{16} are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile,

alkoxy, phenyl, substituted phenyl, where adjacent substituents are taken together to form a ring, and combinations thereof;

X_1 - X_5 are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, where adjacent substituents are taken together to form a ring, and combinations thereof; and

one of X_3 and X_4 is attached to the R_{28} group.

58. The fluorescently labeled reagent according to claim 48 wherein the acceptor dye has the general structure



wherein:

R_1-R_4 are each independently selected from the group consisting of hydrogen, and alkyl or where one or more of the groups of R_1 and R_5, R_2 and R_6, R_3 and R_7, R_4 and R_8 are taken together to form a ring;

R_5-R_{10} are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile, alkoxy,

phenyl, and substituted phenyl, or where two or more of R_5-R_{10} are taken together to form one or more rings;

X_1, X_3 and X_4 are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile, or alkoxy;

X_2 and X_5 are chlorine; and

one of X_3 and X_4 are attached to R_{28}.

59. The fluorescently labeled reagent according to claim 58 wherein R_1 - R_{10}, X_1, X_3 and X_4 are selected to correspond to a dye selected from the group consisting of DR110-2, DR6G-2, DTMR-2, and DROX-2.

60. The fluorescently labeled reagent according to claim 48 wherein the reagent is selected from the group consisting of deoxynucleoside, deoxynucleoside monophosphate, deoxynucleoside diphosphate and deoxynucleoside triphosphate.

61. The fluorescently labeled reagent according to claim 60 wherein the deoxynucleotides are selected from the group consisting of deoxycytosine, deoxyadenosine, deoxyguanosine, and deoxythymidine.

62. The fluorescently labeled reagent according to claim 48 wherein the reagent is selected from the group consisting of dideoxynucleoside, dideoxynucleoside monophosphate, dideoxynucleoside diphosphate and dideoxynucleoside triphosphate.

63. The fluorescently labeled reagent according to claim 48 wherein the dideoxynucleotides are selected from the group consisting of deoxycytosine, deoxyadenosine, deoxyguanosine, and deoxythymidine.

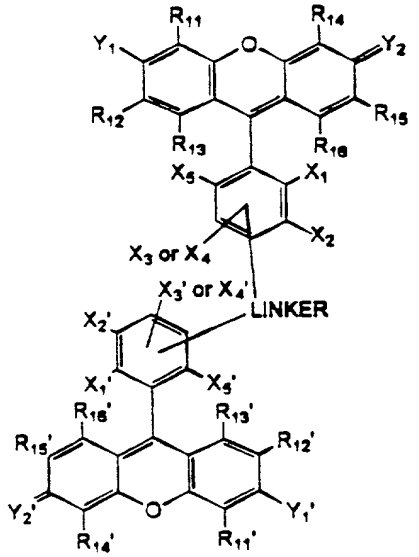
64. The fluorescently labeled reagent according to claim 48 wherein the reagent is an oligonucleotide.

65. The fluorescently labeled reagent according to claim 64 wherein the oligonucleotide has a 3' end which is extendable by using a polymerase.

66. A fluorescently labeled reagent comprising:

a reagent selected from the group consisting of a nucleoside, nucleoside monophosphate, nucleoside diphosphate, nucleoside triphosphate, oligonucleotide and oligonucleotide analog, modified to be linked to an energy transfer fluorescent dye; and

an energy transfer fluorescent dye attached to the reagent, the energy transfer fluorescent dye including a dye having the structure



wherein:

Y_1, Y_1', Y_2 and Y_2' are each independently selected from the group consisting of hydroxyl, oxygen, iminium and amine,

R_{11} - R_{16} and R_{11}' - R_{16}' are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, phenyl, substituted phenyl, where adjacent substituents are taken together to form a ring, and combinations thereof, and

X_1 - X_5 and X_1 ' - X_5' are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, where adjacent substituents are taken together to form a ring, and combinations thereof;

Y_1, Y_2, R_{11} - R_{16}, and X_1 - X_5 are selected to correspond to a donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response;

Y_1 ', Y_2 ', R_{11} ' - R_{16} ', and X_1 ' - X_5 ' are selected to correspond to an acceptor dye which is capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response; and

one of X_3 and X_4 and one of X_3' and X_4' are taken together to form a linker linking the donor to the acceptor dye such that energy is transferred from the donor to the acceptor dye.

67. The fluorescently labeled reagent according to claim 66 wherein the linker has a backbone attaching the donor to the acceptor which is less than 9 atoms in length.

68. The fluorescently labeled reagent according to claim 66 wherein the linker has the general formula $R_{25}Z_3C(O)$ where R_{25} is a C_{1-4} alkyl attached to the donor dye at the X_3 or X_4 substituent, Z_3 is either NH, O or S, C(O) is a carbonyl group and the terminal carbonyl group is attached to the acceptor dye at the X_3' or X_4' substituent.

69. The fluorescently labeled reagent according to claim 66 wherein the linker has the general formula $R_{25}Z_3C(O)R_{26}Z_4C(O)$ where R_{25} is a C_{1-4} alkyl attached to the donor dye at the X_3 or X_4 substituent, R_{26} is a C_{1-4} alkyl, Z_3 and Z_4 are each independently either NH, O or S, C(O) is a carbonyl group and the terminal carbonyl group is attached to the acceptor dye at the X_3' or X_4' substituent.

70. A fluorescently labeled reagent comprising:

a reagent selected from the group consisting of a nucleoside, nucleoside monophosphate, nucleoside diphosphate, nucleoside triphosphate, oligonucleotide and oligonucleotide analog, modified to be linked to an energy transfer fluorescent dye; and

an energy transfer fluorescent dye attached to the reagent, the energy transfer fluorescent dye being selected from the group consisting of: 5 or 6 carboxy TMR-B-CF, 5 or 6 carboxy TMR-F-CF, 5 or 6 carboxy TMR-P-CF, 5 or 6 carboxy TMR-P-CF, 5 or 6 carboxy TMR-A-CF, 5 or 6 carboxy TMR-D-CF, 5 or 6 carboxy TMR-N-CF, 5 or 6 carboxy ROX-CF, CY5-CF, 5 or 6 carboxy TMR-gly-5AMF and 5 or 6 carboxy TMR-5AMF, 5 or 6 carboxy CF-B-TMR-2, 5 or 6 carboxy CFB-DR110-2, 5 or 6 carboxy CFB-DR6g-2, and 5 or 6 carboxy CFB-DROX-2.

71. The fluorescently labeled reagent according to claim 70 wherein the reagent is selected from the group consisting of

deoxynucleoside, deoxynucleoside monophosphate, deoxynucleoside diphosphate and deoxynucleoside triphosphate.

72. The fluorescently labeled reagent according to claim 71 wherein the deoxynucleotides are selected from the group consisting of deoxycytosine, deoxyadenosine, deoxyguanosine, and deoxythymidine.

73. The fluorescently labeled reagent according to claim 70 wherein the reagent is selected from the group consisting of dideoxynucleoside, dideoxynucleoside monophosphate, dideoxynucleoside diphosphate and dideoxynucleoside triphosphate.

74. The fluorescently labeled reagent according to claim 70 wherein the reagent is an oligonucleotide.

75. The fluorescently labeled reagent according to claim 74 wherein the oligonucleotide has a 3' end which is extendable by using a polymerase.

76. A method for sequencing a nucleic acid sequence comprising:

forming a mixture of extended labeled primers by hybridizing a nucleic acid sequence with a fluorescently labeled oligonucleotide primer in the presence of deoxynucleoside triphosphates, at least one dideoxynucleoside triphosphate and a DNA polymerase, the DNA polymerase extending the primer with the deoxynucleoside triphosphates until a dideoxynucleoside triphosphate is incorporated which terminates extension of the primer;

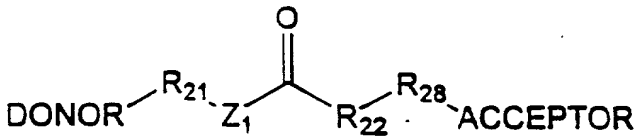
separating the mixture of extended primers; and

determining the sequence of the nucleic acid sequence by fluorescently measuring the mixture of extended primers formed;

the fluorescently labeled oligonucleotide primer including

an oligonucleotide sequence complementary to a portion of the nucleic acid sequence being sequenced and having a 3' end extendable by a polymerase, and

an energy transfer fluorescent dye attached to the oligonucleotide, the energy transfer fluorescent dye having the structure



where

DONOR is a dye capable of absorbing light at a first wavelength and emitting excitation energy in response;

ACCEPTOR is dye which is capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response;

C(O) is a carbonyl group;

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

R_{21} is a C_{1-5} alkyl attached to the donor dye;

R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond or a fused ring structure which is attached to the carbonyl carbon; and

R_{28} includes a functional group which attaches the linker to the acceptor dye.

77. A method for sequencing a nucleic acid sequence comprising:

forming a mixture of extended labeled primers by hybridizing a nucleic acid sequence with a fluorescently labeled oligonucleotide primer in the presence of deoxynucleoside triphosphates, at least one dideoxynucleoside triphosphate and a DNA polymerase, the DNA polymerase extending the primer with the deoxynucleoside triphosphates until a dideoxynucleoside triphosphate is incorporated which terminates extension of the primer;

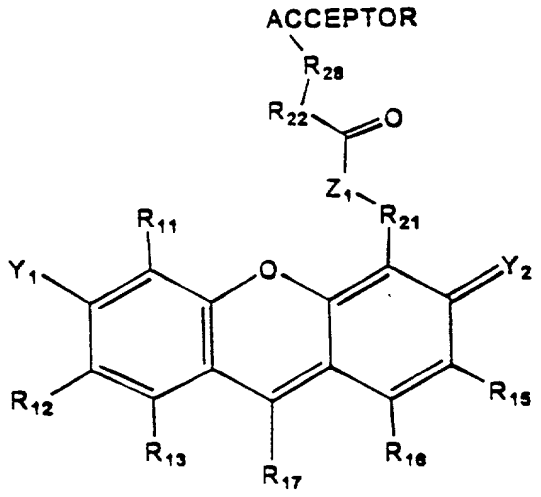
separating the mixture of extended primers; and

determining the sequence of the nucleic acid sequence by fluorescently measuring the mixture of extended primers formed;

the fluorescently labeled oligonucleotide primer including

an oligonucleotide sequence complementary to a portion of the nucleic acid sequence being sequenced and having a 3' end extendable by a polymerase, and

an energy transfer fluorescent dye attached to the oligonucleotide, the energy transfer fluorescent dye having the structure



where

C(O) is a carbonyl group;

Y_1 and Y_2 are each independently selected from the group consisting of hydroxyl, oxygen, iminium and amine;

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

R_{11}-R_{17} are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, phenyl, substituted phenyl, where adjacent substituents are taken together to form a ring, and combinations thereof;

R_{21} is a C_{1-5} alkyl;

R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond or a fused ring structure which is attached to the carbonyl carbon;

R_{28} includes a functional group which attaches the linker to the acceptor dye; and

ACCEPTOR is dye which is capable of absorbing excitation energy emitted by a member of the xanthene class of dyes.

78. A method for sequencing a nucleic acid sequence comprising:

forming a mixture of extended primers by hybridizing a nucleic acid sequence with a primer in the presence of deoxynucleoside triphosphates, at least one fluorescently labeled dideoxynucleoside triphosphate and a DNA polymerase, the DNA polymerase extending the primer with the deoxynucleoside triphosphates until a fluorescently labeled dideoxynucleoside triphosphate is incorporated onto the extended primer which terminates extension of the primer;

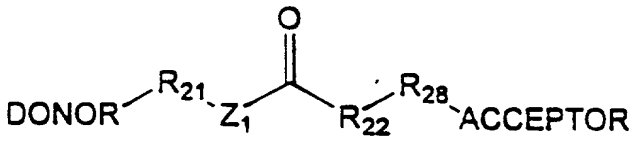
separating the mixture of extended primers; and

determining the sequence of the nucleic acid sequence by detecting the fluorescently labeled dideoxynucleotide attached to the separated mixture of extended primers;

the fluorescently labeled dideoxynucleoside triphosphate including

a dideoxynucleoside triphosphate, and

an energy transfer fluorescent dye attached to the dideoxynucleoside triphosphate, the energy transfer dye having the structure



where

DONOR is a dye capable of absorbing light at a first wavelength and emitting excitation energy in response;

ACCEPTOR is dye which is capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response;

C(O) is a carbonyl group;

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

R_{21} is a C_{1-5} alkyl attached to the donor dye;

R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond or a fused ring structure which is attached to the carbonyl carbon; and

R_{28} includes a functional group which attaches the linker to the acceptor dye.

79. A method for sequencing a nucleic acid sequence comprising:

forming a mixture of extended primers by hybridizing a nucleic acid sequence with a primer in the presence of deoxynucleoside triphosphates, at least one fluorescently labeled dideoxynucleoside triphosphate and a DNA polymerase, the DNA polymerase extending the primer with the deoxynucleoside triphosphates until a fluorescently labeled dideoxynucleoside triphosphate is incorporated onto the extended primer which terminates extension of the primer;

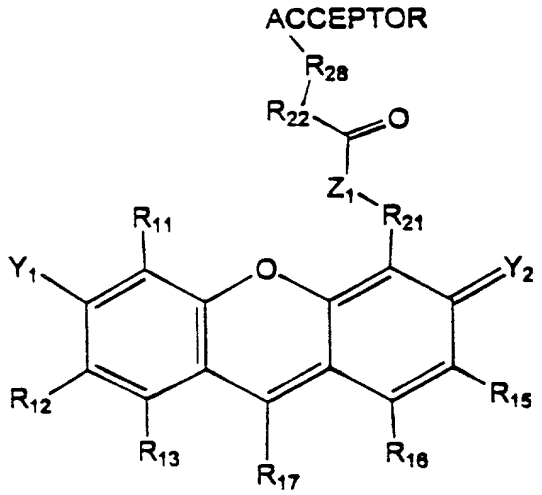
separating the mixture of extended primers; and

determining the sequence of the nucleic acid sequence by detecting the fluorescently labeled dideoxynucleotide attached to the separated mixture of extended primers;

the fluorescently labeled dideoxynucleoside triphosphate including

a dideoxynucleoside triphosphate, and

an energy transfer fluorescent dye attached to the dideoxynucleoside triphosphate, the dye having the structure



where

C(O) is a carbonyl group;

Y_1 and Y_2 are each independently selected from the group consisting of hydroxyl, oxygen, iminium and amine;

Z_1 is selected from the group consisting of NH, sulfur and oxygen;

R_{11}-R_{17} are each independently selected from the group consisting of hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, amino, ammonium, amido, nitrile, alkoxy, phenyl, substituted phenyl, where adjacent substituents are taken together to form a ring, and combinations thereof;

R_{21} is a C_{1-5} alkyl;

R_{22} is a substituent selected from the group consisting of an alkene, diene, alkyne, a five and six membered ring having at least one unsaturated bond or a fused ring structure which is attached to the carbonyl carbon;

R_{28} includes a functional group which attaches the linker to the acceptor dye; and

ACCEPTOR is dye which is capable of absorbing excitation energy emitted by a member of the xanthene class of dyes.